APPLICATION OF QMRA TO EVALUATE THE POTENTIAL HEALTH RISKS ASSOCIATED WITH BACTERIAL CONTAMINATION OF WATERS IN ROYAL BELUM STATE PARK, MALAYSIA

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Abstract

Temenggor Lake located in Royal Belum State Park is an important water resource for indigenous people, an ecosystem for aqua organisms and a place for water recreational activities for visitors. The aim of this research was to use a quantitative microbial risk assessment (QMRA) approach to calculate the probability of potential pathogen infection risk to the visitors and the indigenous community. Thirty-six water samples were collected from the surface waters of Temenggor Lake from recreational water activity and indigenous people activity sites from November 2020 till December 2021. Membrane filtration technique was used for bacterial quantification. The risk was estimated using the calculation of the daily risk of infection, annual risk of infection, and risk of illness per annum and per event. The water quality during sampling periods at year-end showed higher bacterial concentrations related to rainfall events. Bacterial concentrations at visitor water activity areas were within Category A allowing for recreational activities with full body contact while water quality at indigenous people water activity areas were classified as Category B to be used mainly for secondary body contact and hence not allowed for swimming and bathing. Notably, indigenous people's settlement areas recorded a higher risk of illness for both *E. coli* and *Salmonella spp*., mainly at station A03 (Kg. Sungai Tiang). It has been revealed that water quality in indigenous people and visitor water activity areas were significantly different due to being affected by different types of human activity and other factors such as geohydrological conditions, topography, land use, environmental conditions and events. This study provides baseline data to help in future management strategies to ensure the conservation of the natural landscape and economic viability, in addition to the protection of the local community.

Keywords: Water Quality, Health Risks, QMRA, Bacterial Exposure

Introduction

The Royal Belum State Park (RBSP) was originally gazetted as Belum Forest Reserve in 1971 and subsequently regazzetted in 2007 as a State Park in accordance with the Perak State Park Corporation Enactment 2001. RBSP is considered one of the oldest undisturbed protected pristine land masses in Peninsular Malaysia, which is only accessible by boat *via* the southern part of the State Park.

Temenggor Lake located within RBSP is the second-largest artificial lake in Peninsular Malaysia, constructed initially to generate electric power. Temenggor Lake is divided into three zones: Conservation Zone, Recreational Fishing Zone, and Commercial Zone. The Conservation Zone includes upstream areas of Temenggor Lake, such as Sungai Kejar, Sungai Tiang, and Sungai Gadong where approximately 200 indigenous families comprising mainly of the Jahai and Temiar ethnic groups live (1). The lake serves as a water resource to perform their daily chores, such as bathing, drinking, and washing dishes and clothes (2). The probability of faecal pathogens detection in indigenous settlement areas is high, considering defecating activities are carried out openly on the ground and in the lake water. These infectious agents may also be present due to surface runoff of faecal matter from domestic animals and wildlife (3).

Due to their economic potential, many lakes have become popular tourism sites for water-based recreational activities and sports or commercial fishing activities, expanding the water needs and creating conflicting water uses (4). Statistics from the Perak State Park Corporation (5) have shown an increase in tourists from 2,909 in 2006 to 27,805 in 2018. The increase in houseboats and visitors may contribute to the deterioration of water quality in Temenggor Lake in the long term, which could result in a hazard to the user's health. There are minimal sanitation services provided to visitors on land and no uniform structured management on the direct discharge of human excrement into the lake from houseboats as the installation of holding tanks has yet to be deployed.

Water recreation places in Malaysia experiencing water quality deterioration include Kenyir Lake, Chini Lake, and Port Dickson beaches. *E. coli* was detected at the majority of the sampling stations in Kenyir Lake (6); sourced from both animal and human faeces due to improper sewage and wastewater disposal facilities on houseboats. Similarly, the water in Chini Lake was determined to be polluted with total and faecal coliform exceeding the standard count permitted by the Malaysian Department of Environment (7, 8). Studies have also found that Port Dickson beaches had typically high concentrations of *E. coli* (9, 10) including the detection and presence of Meticillin Resistant *Staphylococcus aureus* (MRSA) (11).

Studies on Temenggor Lake's water quality have already been undertaken in the lower lake area, primarily focusing on physicochemical characteristics and elemental analyses (12, 13). Additionally, chemical characteristics of the sediment in Temenggor Lake have shown metal concentrations in lake sediment to be generally low and in the range of natural concentrations (14). However, TSI data of Temenggor Lake showed high total phosphorus content signifying a nutrient-rich environment (15). Despite these results, no thorough investigation of Temenggor Lake's microbiological water quality has been carried out in the state park's upper regions. Therefore, to maintain the longevity of this heritage site, it is vital to monitor this region.

Changes in microbial quality in natural waters arise mainly from agricultural use, sewage discharges, pollution from other human activities, or storm water runoff. In some instances, microbial water quality is strongly influenced by factors such as rainfall that can cause faecal pollution and waterborne disease to rise drastically. Environmental factors such as heavy rains can cause sewage overflows and increase surface water runoff directly into the water, influencing microbiological water quality (16). Individuals who swim or come into contact with water with elevated levels of *E. coli* and other faecal indicator organisms are at an increased risk of becoming sick due to potential exposure to faecal pathogens. Faecal coliform bacteria counts have been reported to be higher in freshwater discharges and human settlements (17) and contribute substantially to the global burden of disease in humans (18). Recreational water generally contains a mixture of pathogenic and non-pathogenic microbes (19), hence evaluation of user health risks is needed to ensure that safety is best when doing water activities.

The potential risks from exposure to contaminated waters can be estimated using quantitative microbial risk assessments (QMRA) (20) which are particularly useful because they can quantify the probability of infection, illness, and morbidity (21). The QMRA approach has been successfully used to model risks from exposures to waterborne pathogens including bacteria and viruses in natural waters (22-24).

Thus, water quality will be measured and assessed using the QMRA approach to evaluate the daily risk of infection, annual risk of infection, and risk of illness per annum and per event associated with exposure to microbiological pollution using pathogenic *E. coli* and *Salmonella* spp., at the settlements of the indigenous people and the houseboat parking areas, which is a place for water recreation activities for visitors. To the best of our knowledge, currently, no data on bacterial exposures and potential health risks have been reported in this study area.

Materials and Methods

Sample collection

Sampling was conducted between November 2020 and December 2021 from six sampling stations within the RBSP covering the indigenous people's water activity areas and the common locations for visitor water activities during houseboat trips. Table 1 shows the GPS coordinates and type of sampling locations for the study. Water samples in the volume of 1 liter were collected using the grab technique from surface depths of approximately 15-30 cm.

Table 1: The sampling locations and GPS coordinates

Station	Location Name	GPS Coordinate	Users
A01	Kg. Sungai Ta'hain	5.747500, 101.390833	Indigenous people
A02	Kg. Klewang	5.680359, 101.409440	
A03	Kg. Sungai Tiang	5.695500, 101.442400	
V01	Houseboat Park Sg. Ko'oi	5.666759, 101.399956	Visitor / Tourists
V02	Houseboat Park Sg. Papan Luar	5.638055, 101.370555	
V03	Houseboat Park Sg. Ruok	5.595114, 101.377868	

Bacterial analysis

The membrane filtration technique was used according to standard procedures for bacterial analysis of water samples

(25, 26). The media that had been used in this study are as follows MI agar (*E. coli*), mEI agar (Enterococci), and Brilliant Green agar (*Salmonella* spp. other than *S. typhi* and *S. paratyphi*).

Several dilution series which are 10 mL, 1 mL, 0.1 mL, and 0.01 mL were prepared by initially transferring 11 mL of sample into 99 mL of sterile Buffered Dilution Water. The samples were filtered through a sterilized 47 mm, 0.45 µm pore size cellulose ester membrane filter, and the filter was placed on a plate of agar in duplicate. Plates were incubated at 35°C for 24 hours (MI agar), 41°C for 24 hours (mEI agar), and 35°C for 48 hours (Brilliant Green agar), respectively. The presence of colonies was counted based on colony appearance characterization. The test results were reported in colony-forming units (CFU) per 100 mL sample and were calculated using the equation below under a fluorescent lamp with a magnifying lens and recorded.

Colony Forming Unit (CFU) = Number of colonies counted/ Volume of filtered sample (ml)

Quantitative Microbial Risk Assessment

Quantitative Microbial Risk Assessment (QMRA) was used to estimate the probability for risk of infection or illness due to exposure to infectious microorganisms. It consists of four steps: hazard identification, dose-response assessment, exposure assessment, and risk characterization. Hazard identification is a qualitative process intended to identify microorganisms of concern whereby in this study, pathogenic *E. coli* and *Salmonella* spp. were selected for the risk of illness assessment. Due to the absence of local public health and microbiological data, thus, the concentration of pathogenic *E. coli* was estimated from published ratios of generic *E. coli* to pathogenic *E. coli* as 1:0.8 (27); hence average results of *E. coli* concentration were multiplied by 0.08 in order to obtain the risk assessment from pathogenic *E. coli*.

Exposure assessment is the magnitude and frequency of exposure to each reference pathogen (28). Required information (location of water activities, exposed population, frequency and duration of activities and volume of water accidentally swallowed) was collected through a field survey and interviews among healthy adult water users. Data was collected from two (2) groups which were indigenous people living within the vicinity of Temenggor Lake who used lake water for bathing and visitors who had water activities with whole-body contact. The exposure dose was calculated using the following mathematical equation (29);

$$D = N \times V_{con} \times f$$

where D is the exposure dose expressed as number of microorganisms ingested (CFU), N is the concentration of the microorganisms in water (CFU per liter), V_{con} is the volume consumed in one exposure (L per day), and f is the frequency of use per day.

Dose-response models were used to estimate the probability of infection resulting from a certain pathogen dose. The probability of infection was estimated using the following equation (30);

$$R_{inf} = 1 - (1 + D / \beta)^{-\alpha}$$

Where R_{inf} is the probability of infection at a certain dose, D is the dose in CFU, α is the model infectivity, and β is the model shape for each organism (31, 32) (Values for α and β are depicted in Table 2). Consequently, daily risk of infection is assumed to be the same for each day of the year, and therefore variability in these parameters is not accounted for.

The risk characterization is a combination of dose-response and exposure assessment data which results from the calculation of the annual risk of infection and risk of illness. The risk of annual infection was determined using the standard equation (33) below

$$R_{inf/y} = 1 - (1 - R_{inf/day})^{n}$$

where $R_{inf/y}$ is the annual risk of infection, $R_{inf/day}$ is the risk of infection per day and n is the number of exposure days in a year. The average number of days per year was set as 183 for the indigenous people group since they were not bathing daily, while four days was set as the maximum day for visitors who temporarily visited.

To determine the annual risk of illness, the following equation was used;

$$R_{ill} = R_{inf/y} \times P_{ill/inf}$$

Where R_{ill} is the annual risk of illness, $R_{inf/y}$ is the annual risk of infection and $P_{ill/inf}$ is the probability of illness per infection (Table 2).

Table 2: Constant parameters for risk calculations

Organism	Parameters
Escherichia coli	$\alpha = 0.2099$
	$\beta = 42.8$
	$P_{ill/inf} = 0.35$
***Salmonella* spp.**	$\alpha = 0.33$
	$\beta = 139.9$
	$P_{ill/inf} = 0.45$

Results

Microbial analyses of surface water

The concentration of *E. coli*, enterococci, and *Salmonella* spp. at Temenggor Lake ranged between 0 – 520 CFU/100 mL, 0-374 CFU/100 mL and 84-42300 CFU/100 Ml, respectively. The highest concentrations of *E. coli* and enterococci were recorded at A03 whereas for *Salmonella* spp. V01 recorded the highest concentration. The lowest concentrations for all three bacteria were recorded at V02. In comparison to the others, the concentration of *Salmonella* spp. appears to be high throughout the sampling period for all stations.

Table 3: The min, max and geometric mean of bacterial concentration

Parameters	Station	Min	Max	Geometric Mean
***E.coli* (CFU/100mL)**	A01	5	40	40
	A02	11	203	203
	A03	32	520	520
	V01	2	46	46
	V02	ND	13	13
	V03	ND	20	20
Enterococci (CFU/100ml0	A01	1	30	11
	A02	3	155	31
	A03	52	374	135
	V01	3	16	8
	V02	ND	17	2
	V03	ND	10	1
***Salmonella* spp. (CFU/100mL)**	A01	245	22200	2289
	A02	222	32050	1907
	A03	898	35700	4102
	V01	166	42300	927
	V02	84	17490	1518
	V03	358	16200	1245

Results of the t-test showing a comparison of microbiological density between the different types of sampling stations are depicted in Table 4. There was a significant difference in concentration of *E. coli* ($t(17) = 3.144$, $p = 0.003$) and enterococci ($t(17) = 2.994$, $p = 0.004$) between the indigenous people's area and visitors' recreational area. Indigenous peoples' area recorded a higher concentration of *E. coli* and enterococci compared to the visitors' recreational area. However, there was no significant difference in the concentration of *Salmonella* spp. between these localities.

Table 4: Comparison between indigenous and visitors' areas

Parameters (CFU/ 100mL)	Indigenous		Visitor		df	t	p
	Mean	SD	Mean	SD			
E. coli	125	154	10	11	17	3.144	0.003
Enterococci	75.9	97.7	5.9	5.3	17	2.994	0.004
***Salmonella* spp.**	7770	10971	5115	10626	17	0.854	0.202

Exposure assessment

The exposure scenario was considered for both indigenous people and visitors to the RBSP. Swallow data was used in the calculation of exposure dose where the volume of water accidentally ingested during swimming or water activities was estimated based on the ranges of 37–52 mL for indigenous people and 61–70 mL for visitors per activity. The average bacterial concentration for each sampling station was used to calculate the exposure dose. The highest exposure dose was for exposure to *Salmonella* spp. for all stations. The lowest calculated exposures were for *E. coli* at A01 and V03. The results of the exposure assessment for all organisms in each station are presented in Table 5.

Table 5: Average exposure dose comparison between indigenous people and visitors

Type of Activity	Station	Exposure Dose (CFU/L/day)		
		E. coli	**Enterococci**	***Salmonella* spp.**
Indigenous people water activity areas	**A01**	6.51E-02	7.46E-01	1.55E+02
	A02	1.79E-01	1.21E+00	7.47E+01
	A03	1.20E+00	9.63E+00	2.93E+02
Visitors recreational water activity areas	**V01**	1.29E-01	1.62E+00	1.87E+02
	V02	1.29E-01	6.91E-01	3.50E+02
	V03	8.36E-02	5.22E-01	3.25E+02

Risk of Illness

The number of exposure days in a year differed between indigenous people and visitors, depending on the population's water activity rate per year. Based on interview sessions with indigenous people, most stated only bathing and washing once every 2 to 3 days except in the dry season hence we assumed the number of exposure days in a year for indigenous people was 183 days. The maximum duration per visit to RBSP was four days, hence we estimated the number of exposure days per year for the visitors to be four.

Station A03 was found to have a higher risk of infection to indigenous people for all organisms tested in a year, whereas Station V01 was found to have a higher risk of infection to visitors per trip. The annual risk of infection by *Salmonella* spp. was much higher for all stations compared to other organisms tested. The estimated annual risk of infection per year among indigenous people and visitors for each organism is shown in Table 6.

Table 6: Annual risk of infection

Type of Activity	Station	Annual risk of infection		
		E. coli	*Enterococci*	*Salmonella* spp.
Indigenous people water activity areas [a]	**A01**	0.0567	0.0270	1.0000
	A02	0.1479	0.0436	1.0000
	A03	0.6539	0.2962	1.0000
Visitors recreational water activity areas [b]	**V01**	0.0025	0.0013	0.6742
	V02	0.0025	0.0006	0.8086
	V03	0.0016	0.0004	0.7951

Estimated exposure is [a]183 days and [b]4 days.

Only *E. coli* and *Salmonella* spp. was used to estimate the risk of illness, as the constant for the probability of illness was unavailable for enterococci. Indigenous people using water from station A03 (Kg Sungai Tiang) were estimated to have a higher risk of illness due to *E. coli* infection in a year (22.9%), compared to those at stations A02 (5.2%) or A01 (2.0%). Visitors who indulged in recreational water activities at all stations were estimated to have a 0.1% risk of illness due to *E. coli* infection per trip.

Indigenous people were estimated to have a 45% risk of salmonellosis per infection due to *Salmonella* spp. for all stations within a year while the estimated risk of salmonellosis among visitors was between 30.3 - 36.4% for an individual per trip. Based on the risk assessment results, it can be said that the risk of illness to indigenous people was the highest. Table 7 represents the risk that an individual will develop an illness per infection in a year or per trip due to exposure.

Table 7: Risk of illness per year/trip among visitor and indigenous people

Type of Activity	Station	*E. coli*		*Salmonella* spp.	
		Risk of illness per year	Risk of illness (%)	Risk of illness per year	Risk of illness (%)
Indigenous people water activity areas	**A01**	0.020	2.0	0.450	45.0
	A02	0.052	5.2	0.450	45.0
	A03	0.229	22.9	0.450	45.0
Visitors recreational water activity areas	**V01**	0.001	0.1	0.303	30.3
	V02	0.001	0.1	0.364	36.4
	V03	0.001	0.1	0.358	35.8

Discussion

The decline in the microbiological quality of the water may expose users to bacteria that can infect them. The most prevalent illnesses include gastroenteritis, nausea, and cramps, primary amoebic meningoencephalitis, which causes inflammation of the brain and meninges, and respiratory illnesses caused by waterborne viruses, such as acute febrile respiratory sickness, pneumonia, and conjunctivitis (34). In essence, the National Lake Water Quality Criteria and Standards (NLWQS) for Malaysian lake water use specify the water quality needed for various protection purposes based on user needs. The criteria are meant to offer the necessary information for making decisions regarding the suitability of lake water for human protection or recreational uses and apply to any lake, dam, reservoir, pond, or other impoundments (35).

Based on the NLWQS, the concentrations of *E. coli* recorded at stations A02 and A03, and enterococci at station A03 are categorized as Category B to be used mainly for secondary body contact recreation such as boating and cruising but are not allowed for swimming and bathing. Nevertheless, the concentration of *E. coli* and enterococci at all visitors' water activity areas were classified as Category A under the NLWQS allowing for primary body contact such as swimming, diving and kayaking. Most of the parameters tested have shown that water quality in both areas was significantly different due to being affected by a different type of activity.

Cabral (34) has stated that the ratio of counts of faecal coliforms to faecal enterococci has been proposed to differentiate between contamination from human and animal sources. The numbers of intestinal enterococci are generally about an order of magnitude lower than *E. coli* in human faeces. In this study, since all stations showed the numbers of enterococci lower than *E. coli* on average, it strengthens the fact that faecal contamination in Temenggor Lake is coming from the excrement of indigenous people related to defecating activity on land. There is a lack of facilities such as toilets and sewage tanks in most settlements, therefore, the community resort to defecating in holes dug into the land, producing underground sewage. Environmental factors such as heavy rainfall cause sewage overflows and waste to runoff into the lake, subsequently influencing microbiological water quality (19). Abia et al. (32) found that the rise of *E. coli* counts in water following sediment disturbance were approximately ten times higher than where sediments were undisturbed.

Furthermore, surface runoff is a significant driver of *Salmonella* load in surface waters. Serovar analysis of natural waters reveal that the *Salmonella* contribution to surface waters was often of mixed human and animal origin, highlighting the role of wildlife animals in water contamination (36). Although *Salmonella* do not reproduce rapidly in the natural environment, they can live for several weeks in water and soil under suitable temperatures, humidity, and pH conditions (37). The durability of

Salmonella might explain why concentrations of *Salmonella* spp. were high throughout the months of sampling at all locations.

Temenggor Lake has confluence basin features that provide a "running-water reservoir" with inflow and outflow channels contributing to higher flushing rates resulting in the dilution of pollutants and microorganisms (38). High concentrations of these organisms from the indigenous people's area flow away to the lower parts of RBSP quickly. In addition, the pollution source from houseboat disposals is considered low, considering the number of houseboat entries is limited compared to the relatively large volume of the lake.

Pathogen concentration in surface waters within the same area varies depending on many factors, including the catchment size, geohydrological conditions, topography, climate, land use, and the associated faecal sources upstream of the sampling location. In this study, the highest concentration of all organisms was recorded during the months of November and December. This condition is related to seasonal influence due to the start of the monsoon in Peninsular Malaysia in November, according to the data for daily rainfall amount of 2020 to 2021 from the Meteorology Department of Malaysia. Rainfall and hydrogeological characteristics of the basin can change the flow of water, which significantly impacts the transport concentration (39).

The QMRA is a relatively recent tool that enables the investigation of various scenarios for people interested in the potential health effects of recreational water consumption. The risk of infection varies with location, type of water activities and duration of exposure (30, 39). However, several assumptions are required and calculations are limited by the pathogens for which dose-response relationships are available. In this study, the water intake rate, location, concentration of pathogens, users' activities, and duration are different playing an essential role in contributing to the risk of infection. In the past, there is no study available in the region that addresses the microbiological quality of lake water and associated risks of infection to indigenous people and visitors.

The indigenous community living in Kampung Sungai Tiang was found to have a higher risk of infection for both *E. coli* and *Salmonella* spp. in a year. This village is the largest located within the RBSP in terms of population where 60 families of more than 400 people live. Russo et al. (40) have mentioned in their study that the risk of illness is associated with both the concentration of pathogens in the water and the degree of contact with those pathogens. Water supply here is mainly pumped lake water, the nearby river water or rainwater as the main resources in daily use including activities of washing clothes and bathing (41).

According to the Centres for Disease Control and Prevention (42), the symptoms of pathogenic *E. coli* infections vary for each person often including severe stomach cramps, diarrheal (often bloody), and vomiting. However, some infections may be very mild, while others are severe or even life-threatening. Studies have shown that high densities of *E. coli* and enterococci recovered from recreational waters strongly correlate with swimming-associated gastrointestinal disease (43, 44). Additionally, there is strong evidence that recreational water activities with a high likelihood of water immersion are associated with an elevated risk of various illnesses (45).

The estimated risk of salmonellosis due to *Salmonella* spp. infection among visitors and the indigenous community was found to be much higher than the risk of infection by *E. coli.* Infected humans can harbour *Salmonella* spp. for considerable periods within the gut without signs of disease. These people can be chronic holders of the bacterium in the gut, and constitute the main reservoir of the bacteria in the environment (46). Salmonellosis is usually characterized by acute onset of fever, abdominal pain, diarrheal, nausea, and sometimes vomiting; with symptoms that are relatively mild and patients making a recovery without specific treatment in most cases (47).

The elderly, infants and immunocompromised individuals are more susceptible to bacterial, fungal, viral, and parasitic infections than healthy individuals and are more likely to develop complications from common infections (42). In this study, the risk of illness was calculated limited to the assumption that visitors and indigenous people were healthy adults.

Currently, the concern is to the minimization of human health risks associated with recreational water use and the protection of water resources outweighs the risk of illness to the indigenous community. Visitors involved in recreational activities involving full body immersion should plan well taking into consideration the local rainfall conditions to minimize potential exposures and protect users. However, since the risk of illness to the indigenous community was higher compared to visitors to the area, actions, and preventive measures need to be taken by the relevant authorities taking into account the causes of the health problems to the indigenous people. Low economic status, lack of basic facilities, health care, and hygiene levels further increase the risk of infection and illness among indigenous people (48). As such, this evidence may inform public health officials of the potential risks and improve the contributing factors in the future.

Conclusion

This study revealed that water activities and recreation in some Royal Belum State Park regions exposed varying degrees of health risk. Most parameters were within an acceptable range for primary contact water activities, however, it was identified that enterococci and *E. coli* at station A03, as well as *Salmonella* spp. in all stations in Temenggor Lake be a cause for concern. The major contributor to the low water quality of Temenggor Lake in RBSP is within the area of indigenous settlements caused by a lack of proper sanitation and sewage systems made worse during the rainy season. The use of QMRA to determine the risk of infection and illness is helpful when epidemiology data is unavailable, mainly for indigenous people who find it difficult to access health facilities and services. Sanitation infrastructure needs to be upgraded in the indigenous settlements to include environmentally appropriate sanitation solutions by the respective authorities.

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Competing interests

The authors declare that they have no competing interests.

Ethical clearance

We obtained approval from the UiTM Research Ethics Committee registered under REC/07/2020 (MR/160).

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